Annex 3

EPIGENETIC REGULATION OF PROTEIN BIOSYNTHESIS APPLIED TO BLUE-GREEN ALGAE IN CULTURE

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The following experiment takes source in a seminar given in 1995 by Joel Sternheimer at the European University of Research.

Results of various experiments on the epigenetic regulation of protein biosynthesis having been exposed, a fruitful discussion arised within the audience. Dr J.P.Gerard pointed out that the results were surely interesting, yet the number of parameters involved in tomato cultures or bread making for example was quite large. It would be advantageous, he said, to study systems as simple as possible so that the number of parameters be reduced. Meanwhile Michel Lempereur, who was interested in pollution problems, offered to provide us with equipment to work in aquatic medium. We thus decided to investigate the application of the epigenetic regulation process to aquatic prokaryotic algae.

We undertook to stimulate the growth of blue-green algae -prokaryotes, genus Anabaena- by epigenetic regulation. Their photosynthetic activity involves in particular pigmentary proteins (cyanins). Thus their biosynthesis is easily observed through color change and oxygen release.
We want to point out that this first attempt of stimulation in an aquatic medium is relatively simple to reproduce. We believe that the results obtained are particularly promising. One may add the fact that it points towards numerous applications

Materials ans methods.

- Dilution of 12 ml of Anabaena variabilis (stock provided by the Ecole Normale Superieure de Paris) in 1500 ml of mineral water.
- Addition of 40 g of dry vegetable manure containing 8%, say 2.6 g, of nitrates as well as 40 g of river pebbles (As suggested by Vincent Bargoin this would provide the solution with trace elements).
- Adaptation time to the cultures medium: four days.
- Transfer of 750 ml of the solution in two vats subjected to natural enlightment. This setting in culture started on the 30th of April.

Musical diffusions.

The music has been diffused in one of the vats, by mean of an aquatic speaker Altec UW-30, while the other vat served as a control.
The proteins transcripted in musical sequences were the following:

- TAPE I (45 mn)

NIF H of Anabaena v. (five times)
Allophycocyanin of Anabaena v. (three times)
Plastocyanin of Anabaena v. (three times)
Nitrate reductase of Chorella s. (three times)
PSI photosystem protein of Anabaena v. (Three times) (*)
Ferredoxin of Anabaena v. (five times)
protein 35 K of Anabaena v. (eight times) (*)

- TAPE II (15 mn)

Allophycocyanin of Anabaena v. (two times)
Plastocyanin of Anabaena v. (two times)
PSI photosystem protein of Anabaena v. (three times) (*)
Ferredoxin of Anabaena v. (four times)
Protein 35 K of Anabaena v. (eight times)

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- TAPE III (15 mn)
Ferredoxin of Anabeana v. (two times)
NIF H of Anabeana v. (three times)
NIF A of Anabeana v. (three times) (*)
NIF D of Anabeana v. (three times) (*)
Nitrate reductase of Chlorella s. (three times)
Protein 35 K of Anabeana v. (two times). (*)

The transcriptions had been realized by J.Sternheimer on a sampler Casio SK1 apart from those labelled (*) which were made by P.Ferrandiz on a "One Key Play" software written by Sylvie Guillou and Fabrice Ocelli (INSERM St-Anne, Paris). The rate of the transpositions is tuned so as to make their length correspond to the photoperiods of the micro-organisms.

Tape I was played twice a day, from the 30th of April to the 5th of May. Then from the 7th to the 10th of May TAPE II was played in the morning while TAPE III was played in the evening.
During this period the viability of the micro-organisms was regularly controlled; Samples were drawn from the cultures and then checked under a microscope.

Results

- Evolution of the coloration of cultures (Fig. 1).
Once poured in the vats the solutions looked opaque (after tossing). This was due to the manure mentionned above, the dilution rate of the original stock but also to the spread of a fibrous contaminant which was not characterized.
From the 2nd day of listening the musical vat presented a greater proportion of suspending matter than the control one. However this trend reversed itself by the 4th day. We therefore assumed that the musical exposures had been too long and we decided to abort the diffusion of Tape I.
Instead Tapes II and III have been used. We then observed on May 8th that the tint of the cultures in the musical vat displayed a green blue coloration more pronounced than those in the control vat (Fig. 2). This trend kept increasing up to the end of the experiment.

- Oxygen release.
Ten days after the end of the period of diffusion the musical cultures became caracterized by a proliferation of bubbles at the surface (Fig. 3). Since these bubbles had the property to revive the flame of a lighted match which was put close by, we concluded they contained oxygen. On May, 24th there were about 70 surface bubbles and on the 28th they were 130 (Fig. 4). We point out that the maximum number of visible bubbles observed in the control vat is 8. Hence there is more than a factor 16 between the two cultures with respect to oxygen release. In fact the medium of the musical culture was saturated with oxygen at the end of the observation time.
Clearly this is correlated to an increase of the photosynthetic activity in the musical vat. It indicates that while the oxygen was released some carbonated composites have been fixed (Fig. 5, taken six months later).

Thus this particular application of the epigenetic regulation process led to an interesting depollutive system. This should beget further interests.